Book-Review

Antibiotics and Drug Pharmacology

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Introduction

Drugs are chemicals which when administered to living organisms produce a biological effect. The term antibiotic was derived from the word "antibiosis", which literally means "against life". Antibiotics, in the past, were considered to be organic compounds produced by one microorganism which are toxic to other microorganisms by selectively killing or inhibiting the growth of other microorganisms [1]. In modern terms, this definition includes antimicrobials produced through synthetic means partly (semi-synthetic) or wholly (synthetic) [2].

Some antibiotics are able to kill bacteria completely while some only inhibit their growth. Bactericidal are those that kill bacteria while bacteriostatic are those that inhibit bacterial growth [3]. In September 1928, the first antibiotics (Penicillin) was accidentally discovered from a soil inhabiting fungus *Penicillium notatum* by late Sir Alexander Fleming, an English Bacteriologist, but its discovery was first reported in 1929 [4], it was purified in 1940 by Florey and Chain, who purified it by freeze drying and a Nobel prize was won in 1945. Clinical trials were first conducted on humans in 1940 and during World War II, penicillin saved 12-15% of lives [1,5].

Selman Waksman discovered Streptomycin in 1943 which is active against all Gram-negatives and it is the first antibiotic active against *Mycobacterium tuberculosis*. Most severe infections are caused by gram-negatives and *Mycobacterium tuberculosis.*

Basic anatomy of bacterial cell

The cytoplasmic membrane of Gram-positive bacteria is surrounded by a tough and rigid mesh called the cell wall. The cell wall is a tough layer that gives bacterium its shape, it also shield it from both mechanical and from osmotic stresses. But the Gram-negative bacteria have a thin cell wall that is surrounded by another membrane called outer membrane (OM) aside from the cytoplasmic membrane. The OM is another protective layer which prevents many substances from entering into the bacterium, but it contains some channels called porins. The porins allow the entry of various molecules into the bacterium such as drugs [6]. The periplasm is the space between the outer membrane and cytoplasmic membrane. The cytoplasmic membrane prevents ions from flowing into or out of the cell; this maintains the cytoplasmic and bacterial components in a well defined space. Gram staining is based on the ability of bacteria cell wall to retain crystal violet dye during solvent treatment. The cell walls for Gram-positive microorganisms have a higher peptidoglycan and lower lipid content than Gram-negative bacteria [7].
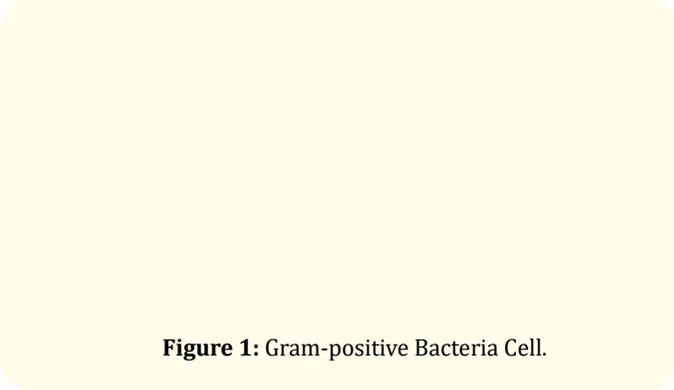
Figure 1: Gram-positive Bacteria Cell.
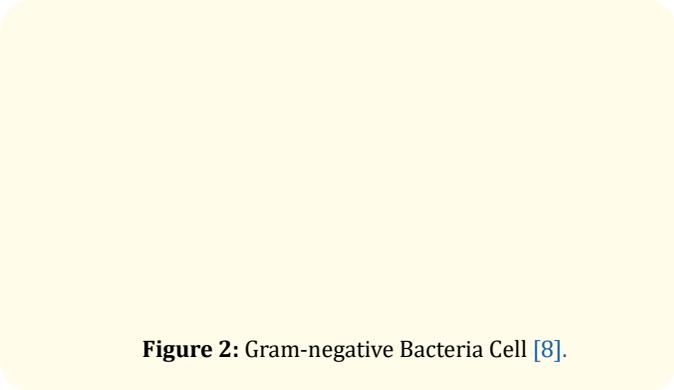
Figure 2: Gram-negative Bacteria Cell [8].

Common Gram-positive organisms are *Streptococcus, S. aureus, Bacillus anthracis, Clostridium botulinum*. Common Gram-negative organisms are *Klebsiella pneumonia, Shigella, Yersinia pestis, Salmonella typhimurium, Salmonella enteriditis.*

Classification of antibiotics

There are several ways of classifying antibiotics but the most common classification are based on their molecular structures, spectrum of activity and mode of action [8]. The uncommon classification includes: route of administration, which can be injectable, topical and oral). The allergic-potential side effects, toxicity and effectiveness of antibiotics in the same structural class will show the same pattern.

Classification based on molecular structures

Some common classes of antibiotics based on their molecular or chemical structures include Beta-lactams, Tetracyclines, Macrolides, Quinolones, Sulphonamides, Aminoglycosides, Oxazolidinones and Glycopeptides [9-11].

Figure 3: Beta-lactam family [2].

Classification based on mechanisms of action for antibiotics

There are differences in cell target of antibiotics and these have led to the difference in mechanism of action. The mechanisms of action of some common antibiotics will be discussed under this section.

Antibiotics targeting cell wall

- Inhibition of peptidoglycan cross-linking (beta-lactams)
- Inhibition of peptidoglycan synthesis (vancomycin).

The peptidoglycan, also called murein in older sources, of the cell wall surrounding the bacterial cells are made of long sugar polymers. Peptidoglycan has some cross-linking peptide bonds known as β-(1-4) –N– acetyl Hexosamine [13,14]. The bacteria must necessarily synthesize the peptidoglycan for it to stay alive and they do this by the activity of Penicillin Binding Proteins (transpeptidases and transglycosylase). The glycan strands of existing peptidoglycan molecule are extended by these two enzymes by adding disaccharide pentapeptides and they also cross-link strands of immature peptidoglycan units [15]. The enzyme transglycosidases catalyzes the cross-linking of the glycan strands by of the peptidoglycan and there is an extension of the peptide chains from the polymers sugar

and form cross links, that is, one peptide to another [16]. The D-alanyl-alanine portion of peptide chain is cross linked by glycine residues in the presence of penicillin binding proteins (PBPs) [17], this strengthens the cell wall. The Beta-lactams and the glycopeptides inhibit cell wall synthesis and peptidoglycan cross-linking.

Beta-lactam antibiotics

The β-lactam majorly targets the Penicillin Binding Proteins. The β-lactam ring is an analog of the D-alanyl D-alanine portion of peptide chain that binds with the PBPs. The β-lactam mimics D-alanyl D-alanine and is bounded by PBP. Therefore, the PBPs are not available for the synthesis of new peptidoglycan and this disruption leads to the lyses of bacterium cell wall and ultimately the death of the bacteria [18]. Drugs like penicillin (examples are penicillin and amoxicillin), cephalosporin (examples are cephalexin and cefdinir) and carbapenem are able to block the cross-linking of peptidoglycan units by inhibiting the peptide bond formation catalyzed by the penicillin Binding Proteins [19]. Examples of Beta-lactam antibiotics with increased activity are amoxicillin/clavulanate, ceftazidime/avibactam.

Glycopeptides

The glycopeptides binds to D-alanyl D-alanine portion of peptide side chain of the precursor peptidoglycan subunit. The drug molecule vancomycin prevents binding of this D-alanyl subunit with the Penicillin Binding Proteins, and hence inhibits cell wall synthesis [18,20]. Most of the antibiotics that belong to the glycopeptide class of antibiotics (e.g. vancomycin) inhibit the synthesis of peptidoglycan which leads to bacterial growth inhibition. This is done by binding themselves to peptidoglycan units, as well as blocking the penicillin binding proteins (transglycosylase and transpeptidase) activities [16].

Disruption of cell membrane
Polymyxins

These classes of antibiotics which damages cell membranes of bacteria are specific in each microbial group because of the differences in the types of lipids in their cell membranes. Daptomycin, for example, depolarizes calcium-dependent membrane, which leads to the ceasing of the synthesis of macromolecules and disruption of the cellular membrane in bacteria [21]. The effective binding of polymyxins to the lipid moiety of the lipopolysaccharide in the bacterial cell causes disintegration of bacterial cell membrane [22]. The disintegration of the cell membrane is used against Gram-negatives for local treatment and it has a narrow spectrum.

Inhibition of protein synthesis

- At 30S ribosomal subunit (aminoglycosides, tetracyclines)
- At 50S ribosomal subunit (macrolides, chloramphenicol).

The central dogma of molecular biology is the transcription of DNA into RNA and the translation of RNA into protein. First, the genetic information in the bacterial DNA is transcribed into an RNA

molecule called messenger RNA (mRNA) through transcription. Then, ribosome synthesizes proteins present in mRNA through translation. The biosynthesis of Protein is catalyzed by cytoplasmic factors and ribosomes. The 30S and 50S ribonucleoprotein subunits make up the bacterial 70S ribosome [23]. Either of these two ribonucleoprotein subunits is the target of the antimicrobials that inhibit protein biosynthesis [24,25]. Some of these antimicrobials are discussed below;

Inhibitors of 30S subunit

- **Aminoglycosides:** The aminoglycosides (AGs) gain access into the bacterium by forming pores on the outer membrane of the bacterium. Recall, like charges attract while unlike charges repel, the AGs are positively charged and attached to the outer membrane which is negatively charged. This leads to the formation of the large pores and ultimately allows the antibiotic to penetrate inside the bacterium. For the antibiotics to reach its target, which is the bacterial ribosome, it needs to pass through cytoplasmic membrane which requires energy dependent active bacterial transport mechanism, this requires oxygen and an active proton motive force (PMF). Because of these requirements, AGs work under aerobic conditions and have poor activity against anaerobic bacteria. These antibiotics have synergism with the antibiotics that inhibit cell wall synthesis (e.g. β-lactam and glycopeptides) because this allows greater penetration of AGs within the cell and at low dosages. AG's interact through hydrogen bonds with the 16S ribosomal RNA (rRNA) of the 30S subunit near the A site. This leads to the misreading and premature termination of translation of mRNA [7].
- **Tetracyclines:** Tetracyclines (such as tetracycline, doxycycline, chlortetracycline or minocycline) act upon the conserved sequences of the 16S rRNA of the 30S ribosomal subunit and prevent the binding of transfer RNA (tRNA) to the A site of the ribosome [23,26].

Inhibitors of 50S subunit

- **Chloramphenicol:** It acts upon the conserved sequences of the peptidyl transferase cavity of the 23S ribosomal RNA (rRNA) of the 50S subunit. Hence, it inhibits the protein synthesis by preventing binding of transfer RNA (tRNA) to the A site of the ribosome [23,24].
- **Macrolides:** They target the conserved sequences of the peptidyl transferase center of the 23S rRNA of the 50S ribosomal subunit at translocation (early stage of protein synthesis). This action results in the premature detachment of the incomplete peptide chains. Lincosamides, streptogramins B and Macrolides show similar mechanism of action [7]. Examples of macrolides are azithromycin and erythromycin.
- **Oxazolidinones:** Oxazolidinones affect protein synthesis at several stages; they suppress 70S inhibition and interact

with peptidyl-t-RNA and also inhibit protein synthesis by binding to 23S rRNA of the 50S subunit. Linezolid is a recently approved member of novel class of antibiotic of this group which is completely synthetic [27,28].

Inhibition of nucleic acid

- Inhibition of folic acid synthesis (sulphonamides, trimethoprim)
- Inhibition of DNA gyrase (fluoroquinolones)
- Inhibition of RNA synthesis (rifampin).

Folic acid metabolism inhibitors

Some antibiotics (e.g. sulphonamides and trimethoprim) mimic a substrate needed for cellular metabolism of bacteria, that is, they are analog of this substrate. This makes bacterial enzymes to react or attach themselves to the antibiotic instead of the normal substrate. Tetrahydrofolate is required for the synthesis of folic acid in bacterial cells; sulphonamides mimic this substrate (Tetrahydrofolate) [29]. Folic acid is vital in the metabolism of nucleic acid and amino acids; for this reason, sulphonamides ultimately disrupt the production of nucleic acids (DNA and RNA) and amino acids [29]. Each of these drugs inhibits distinct steps in folic acid metabolism. There is a reduced mutation rate for resistance against these drugs by the bacterium when sulpha drugs are combined with trimethoprim, because each of the drugs act on a distinct step of the biosynthetic pathway of folic acid [23]. Sulfonamide has higher affinity for the enzyme, dihydropteroate synthase than p-amino benzoic acid which is the natural substrate, so it inhibits in a competitive manner. Trimethoprim inhibits the enzyme dihydrofolate reductase at a later stage of folic acid synthesis [23].

Quinilones

Bacterial DNA gyrase is inhibited by fluoroquinolones (FQ). Bacterial DNA gyrase is an enzyme that nicks the double stranded DNA, then introduces negative supercoils and reseals the nicked ends. The DNA gyrase has two A subunits and two B subunits. The nicking of DNA is done by the A subunit, the negative supercoils are introduced by B subunit and the nicked ends are resealed by the A subunit. This process prevents the excessive positive supercoiling of the strands when they separate so that transcription or replication can occur. The A subunit is binded with high affinity by FQ which interferes with the subunit's strand cutting and resealing function. But the main target of FQ in Gram-positive bacteria is topoisomerase IV. The topoisomerase IV nicks and separates daughter DNA strand after DNA replication. Greater affinity for this enzyme by the FQ may confer higher potency against Gram-positive bacteria. Instead of DNA gyrase or topoisomerase IV in the bacterial cell, mammalian cells possess topoisomerase II, which has very low affinity for FQ, this leads to low toxicity to mammalian cells [23,26].

Figure 4: Antibiotic target sites [8].

Pharmacokinetics of antibiotics

Pharmacokinetics (PK) refers to the study of concentration changes of a drug over a period of time. After the administration of a drug, drugs undergoes ADME (absorption, distribution, metabolism and excretion) processes that condition the kinetics of the drug and the concentration-time profile, which may be characterized by the PK parameters, such as total body clearance, volume of distribution, protein binding or bioavailability [30,31].

Aminoglycosides

Aminoglycosides route of administered is majorly via parenteral except cases of intestinal infections or indication for decontamination. aminoglycosides upon absorption into the bloodstream, they have low volume of distribution (<0.3 L/kg) thus aminoglycosides are mainly distributed in blood plasma. In the blood, aminoglycosides bind weakly to plasma protein (about only 30%) and possess a plasma elimination half-life of approximately two hours. They are excreted extensively via kidney (renal excretion) in urine in the unchanged biological active form (only <10% is biotransformed). But small fraction was found to be eliminated through bile (0.5-2% of the administered dose). The PK of elimination is independent of the dose and route of administration. Aminoglycosides are distributed poorly into tissues due to poor diffusion, but they are well distributed into peritoneal, pleural and pericardial fluid and synovial effusions due to good diffusion. In the above aforementioned fliuds, aminoglycosides attain a concentration of 25 to 50% of levels in serum; renal accumulation occurs particularly in the cortex [32].

Tetracycline

Tetracyclines are administered via oral route, while doxycicline and minocycline can be given either orally or intravenously. Tetracycline drugs are distributed in tissues based on their lipophilicity. In the blood stream, they bind moderately to plasma protein in the range of about 20% to 60%. Tetracyclines administered via oral route are absorbed in the stomach and proximal small intestine. Oral bioavailability of these compounds is relatively high, ranging from 75% to 100%. Food reduces the absorption of both tetracycline and doxycycline up to 50%. They have plasma elimination Half-life of 8-25 hours. They are eliminated by both hepatic and renal mechanisms, they are filtered by the gomerulus but due to their high lipophilicity, they are reabsorbed. Tetracycline class are not effectively dialyzed, thus dose adjustment is not necessary in patients with renal impairment [33].

Macrolide

Macrolides are administered via oral route. After oral administration, they are readily absorbed from the gastrointestinal tract if not inactivated by gastric acid. Macrolide have high bioavailability. They also possess high volume of distribution (1–2.5 L/kg) which reflects their extensive tissue penetration. In the bloodstream, 75% of the administered dose is bounded to plasma Proteins. They actually accumulate within many cells, including macrophages, in which they may be ≥20 times the plasma concentration. Macrolides tend to concentrate in the spleen, liver, kidneys, and particularly the lungs. They enter pleural and peritoneal fluids but not the cerebrospinal fluid (only 2–13% of plasma concentration unless the meninges are inflamed). They concentrate in the bile and milk. Macrolide are inactivated by metabolic activities of the liver but the relative proportion of the drug inactivated by metabolism depends on the route of administration and the particular antibiotic. For example, after oral administration of erythromycin, about 80% of the administered dose undergoes metabolic inactivation, whereas tylosin appears to be eliminated in an active form. Macrolide antibiotics and their metabolites are excreted mainly in bile (>60%) and often undergo enterohepatic cycling. Urinary clearance may be slow and variable (often <10%) but may represent a more significant route of elimination after parenteral administration [34].

β-lactams Antibiotics

- **Penicillin's:** Penicillin's are administered mainly via oral route. After oral administration, penicillin's are absorbed in the stomach and proximal small intestine. In the bloodstream, penicilins have different values of protein binding; with the exception of piperacillin and clavulanic acid that are not orally absorbed, the gastrointestinal absorption is >50% for almost all drugs considered. They are minimally metabolized and the main route of elimination is renal excretion as parent drug [35].

- **Cephalosporins:** Cephalosporins after administration, while in the bloodstream, bind to plasma proteins in different degrees. The reported value for protein binding range from 6% for cephradine to 92% for cefazolin. Cephalosporins generally distribute well into the lung, kidney, urine, synovial, pleural, and pericardial fluids. Penetration into the CSF of some third generation cephalosporins (cefotaxime, ceftriaxone, and ceftazidime) is adequate to effectively treat bacterial meningitis. Cephalosporins are extensively elimi-

nated from the body via the kidney, though a few exceptions include cefoperazone and ceftriaxone which have significant biliary elimination. Biliary excretion of cephalosporins is highly dependent on molecular weight in rats: less than 15% of the dose is excreted into the bile for cephalosporins with a molecular weight of less than 450, but those with a molecular weight of more than 450 exhibit 15 to 100% recovery in bile. In addition, their elimination pathway is mainly excretion into bile and/or urine with minimal metabolism in the body [35].

Resistance to antibiotics

Antimicrobials have helped us deal with disease, but on the other hand, the improper use of these antimicrobials has created new difficulties. Antibiotic resistance is the ability of bacteria to protect themselves against the effects of an antibiotic. The appropriate and inappropriate use of antibiotics the past years has lead to increased occurrence and spread of bacteria that are resistant to antibiotics. Resistance against antibiotics can either be natural or acquired.

The under listed human factors has contributed to the development of resistance against antibiotics by bacteria; the prescribing antibiotics too often, too long therapy or too low dose, the stop of medication before completing the therapy, usage of antibiotics in animal husbandry and the spread of resistant hospital strains due to unhygienic lifestyle. The following factors can be considered in limiting resistance; constant exposure to high levels of antibiotic, use of multiple antibiotics and restricted use of antibiotics [36-50].

The following are the common mechanism of antibiotic resistance;

1. Alteration of Targets, this usually affects ribosomes.
2. Alteration of Membrane Permeability, this is caused by the change in the receptor that binds the drug.
3. Development of Enzymes that inactivate antibiotics such as beta-lactamase, aminoglycoside- modifying enzymes and chloramphenicol acetyltransferases. The first β-lactamase was identified in 1940 [7].
4. Efflux pumps; Membrane proteins of Gram negatives that pump out drug
5. Alteration of Metabolic Pathway; Development of alternate metabolic pathway(s).

Below is a table showing the summary of the resistance mechanism of different antibiotics.

Table: Resistance mechanism of individual antibiotics (Garima *et. al.*, 2017).

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